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5.0 Accuracy of the BG1Luc ER TA

This section discusses the accuracy of the BG1Luc ER TA in the multi-laboratory validation effort. Accuracy is evaluated by assessing:

- Concordance: The closeness of agreement between a test method result and a reference value.
- Sensitivity: The proportion of all positive substances that are classified correctly.
- Specificity: The proportion of all negative substances that are classified correctly.
- False positive rate: the proportion of true negative substances that are falsely identified as positive.
- False negative rate: the proportion of true positive substances that are falsely identified as negative.

Each of these variables can be calculated using a simple two-by-two table as follows: concordance ([a+d]/[a+b+c+d]), sensitivity (a/[a+c]), specificity (d/[b+d]), false positive rate (b/[b+d]), and false negative rate (c/[a+c]) (see **Table 5-1**).

Table 5-1 Template for Concordance Analysis

		New Test Outcome		
		Positive	**Negative**	**Total**
Reference Test Classification	Positive	a	c	a+c
	Negative	b	d	b+d
	Total	a+b	c+d	a+b+c+d

The BG1Luc ER TA was evaluated for its ability to correctly identify estrogen receptor agonists and antagonists. For this analysis, test substance classification (Positive or Negative for ER agonist/antagonist activity) obtained during the validation study was compared to the classification of the same substance based on a preponderance of published data. Positive or negative classifications based on BG1Luc ER TA data were based on the majority classification assigned using results from each of the three participating laboratories. For example, if a substance tested positive at one laboratory, but negative in the other two, the overall classification would be negative for the purposes of the accuracy calculations. Substances that failed to meet the decision criteria for either a positive or negative response defined in **Section 2.12.3** are considered "inadequate" for analysis. The classification of data as "inadequate" is due to poor data quality, and would normally require retesting. However, this classification system was developed after testing was complete and therefore these substances were excluded from the accuracy analyses described herein.

5.1 Substances Used for Accuracy Analysis

As detailed in **Section 3.2**, NICEATM completed a comprehensive literature review of available *in vitro* data to identify substances that could be considered unequivocally positive or negative for ER agonist or antagonist activity. A total of 48 unique reference substances were considered in the evaluation of test method accuracy. Separate lists were generated for evaluating accuracy based on agonist (42 substances; 33 Positive, 9 Negative) and antagonist (25 substances; 3 Positive, 22 Negative) activity. There were 19 substances common to both reference lists.

The list of 42 reference substances used to evaluate test method accuracy for ER agonist activity is provided in **Table 5-2**. Of these 42 substances, 7 (17%) had "inadequate" testing results and were therefore excluded from the analysis, leaving 35 (28 Positive, 7 Negative) substances for evaluation. The seven substances for which BG1Luc ER TA agonist test method data were inadequate were: 5∝-dihydrotestosterone, clomiphene citrate, flutamide, p,p'-DDE, procymidone, resveratol, and tamoxifen. These seven substances represent eight chemical classes (two cyclic hydrocarbons, and one each of an amide, amine, carboxylic acid, halogenated hydrocarbon, heterocyclic compound, polycyclic compound, and steroid) and five product classes (four pharmaceuticals and one each of a fungicide, natural product, pesticide intermediate, and veterinary agent). The diversity of chemical and product classes indicates that no one category or class is overrepresented with "inadequate" data. It should be emphasized that the "inadequate" classification is usually a result of poor data quality, and would normally require retesting. However, this classification system was developed after testing was complete and retesting of these substances was therefore not possible.

The list of 25 reference substances used to evaluate test method accuracy for ER antagonist activity is provided in **Table 5-3**. Definitive classifications (Positive or Negative) were obtained for all 25 substances tested, thereby allowing all substances to be used for the assessment of antagonist accuracy.

Table 5-2 42 ICCVAM-Recommended Substances for Evaluation of ER Agonist Accuracy

Substance	CASRN	Classification[a]				
		ICCVAM Consensus	BG1Luc ER TA Consensus	XDS	ECVAM	Hiyoshi
17∝-Estradiol	57-91-0	POS	POS	POS (1/1)	POS (3/3)	POS (2/2)
17∝-Ethinyl estradiol	57-63-6	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
17ß-Estradiol	50-28-2	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
19-Nortestosterone	434-22-0	POS	POS	POS (1/1)	NT	NT
4-Cumylphenol	599-64-4	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
4-*tert*-Octylphenol	140-66-9	POS	POS	I (1/1)	POS (1/1)	POS (2/2)

Substance	CASRN	Classification[a]				
		ICCVAM Consensus	BG1Luc ER TA Consensus	XDS	ECVAM	Hiyoshi
5∝-Dihydrotestosterone	521-18-6	POS	I	I (1/1)	I (1/1)	POS (1/1)
Apigenin	520-36-5	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Atrazine	1912-24-9	NEG	NEG	NEG (3/3)	POS (3/3)	NEG (3/3)
Bicalutamide	90357-06-5	NEG	NEG	NEG (1/1)	NT	NT
Bisphenol A	80-05-7	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
Bisphenol B	77-40-7	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
Butylbenzyl phthalate	85-68-7	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
Chrysin	480-40-0	POS	POS	POS (2/2)	NT	NT
Clomiphene citrate	50-41-9	POS	I	I (1/1)	NEG (1/1)	POS (1/1)
Corticosterone	50-22-6	NEG	NEG	NEG (3/3)	POS (3/3)	NEG (3/3)
Coumestrol	479-13-0	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Daidzein	486-66-8	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Dicofol	115-32-2	POS	POS	POS (1/1)	NEG (1/1)	POS (1/1)
Diethylstilbestrol	56-53-1	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
Estrone	53-16-7	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Ethyl paraben	120-47-8	POS	POS	I (1)	POS (1/1)	POS (1/1)
Fenarimol	60168-88-9	POS	POS	POS (1/1)	NT	NT
Flutamide	13311-84-7	NEG	I	I (1)	NT	NT
Genistein	446-72-0	POS	POS	POS (3/3)	POS (3/3)	POS (4/4)
Hydroxy Flutamide	52806-53-8	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Kaempferol	520-18-3	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Kepone	143-50-0	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
L-Thyroxine	51-48-9	POS	NEG	NEG (1/1)	NT	NT
Linuron	330-55-2	NEG	NEG	NEG (1/1)	NT	NT
meso-Hexestrol	84-16-2	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Methyl testosterone	58-18-4	POS	POS	POS (3/3)	POS (1/1)	POS (2/2)
Norethynodrel	68-23-5	POS	POS	POS (2/2)	POS (1/1)	POS (2/2)
o,*p*'-DDT	789-02-6	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)
p-n-Nonylphenol	104-40-5	POS	POS	POS (3/3)	POS (3/3)	POS (2/3)
p,*p*'- Methoxychlor	72-43-5	POS	POS	POS (1/1)	POS (1/1)	POS (2/2)
p,*p*'-DDE	72-55-9	POS	I	I (1/1)	I (1/1)	NEG (1/1)
Phenobarbital	50-06-6	NEG	NEG	NEG (1/1)	NEG (1/1)	NT
Procymidone	32809-16-	NEG	I	I (1/1)	NT	NT

Substance	CASRN	Classification[a]				
		ICCVAM Consensus	BG1Luc ER TA Consensus	XDS	ECVAM	Hiyoshi
	8					
Resveratrol	501-36-0	POS	I	POS (1/1)	I (1/1)	NEG (1/3)
Spironolactone	52-01-7	NEG	NEG	NEG (1/1)	NT	NT
Tamoxifen	10540-29-1	POS	I	I (1/1)	I (1/1)	POS (1/1)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation;.I = indequate; NEG = negative; POS = positive; NT = Not tested; XDS = Xenobiotic Detection Systems, Inc.

[a]Number in parentheses represents test results (POS, NEG, or I) over the total number of acceptable trials.

Table 5-3 25 ICCVAM Recommended Substances for the Evaluation of ER Antagonist Accuracy

Substance	CASRN	Classification[a]				
		ICCVAM Consensus	BG1Luc ER TA Consensus	XDS	ECVAM	Hiyoshi
17α–Ethinyl estradiol	57-63-6	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
4-Hydroxytamoxifen	68047-06-3	POS	POS	POS (1/1)	I (2/2)	POS (1/1)
5α-Dihydrotestosterone	521-18-6	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Apigenin	520-36-5	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (4/4)
Bisphenol A	80-05-7	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Butylbenzyl phthalate	85-68-7	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (4/4)
Chrysin	480-40-0	NEG	NEG	NEG (1/1)	NT	NT
Coumestrol	479-13-0	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Daidzein	486-66-8	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Di-*n*-butyl phthalate	84-74-2	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Dicofol	115-32-2	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Diethylhexyl phthalate	117-81-7	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Diethylstilbestrol	56-53-1	NEG	NEG	NEG (1/1)	NEG (1/1)	POS (1/1)
Genistein	446-72-0	NEG	NEG	NEG (3/3)	NEG	NEG (3/3)

Substance	CASRN	Classification[a]				
		ICCVAM Consensus	BG1Luc ER TA Consensus	XDS	ECVAM	Hiyoshi
					(3/3)	
Kaempferol	520-18-3	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Kepone	143-50-0	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Mifepristone	84371-65-3	NEG	NEG	NEG (1/1)	NT	NT
Norethynodrel	68-23-5	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
o.p'-DDT	789-02-6	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (3/3)
p-n-Nonylphenol	104-40-5	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (3/3)
p.p'-DDE	72-55-9	NEG	NEG	NEG (1/1)	NEG (1/1)	NEG (1/1)
Progesterone	57-83-0	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (3/3)
Raloxifene HCl	82640-04-8	POS	POS	POS (1/1)	POS (1/1)	POS (1/1)
Resveratrol	501-36-0	NEG	NEG	NEG (3/3)	NEG (3/3)	NEG (3/3)
Tamoxifen	10540-29-1	POS	POS	POS (3/3)	POS (3/3)	POS (3/3)

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; ECVAM = European Centre for the Validation of Alternative Methods; Hiyoshi = Hiyoshi Corporation;.I = indequate; NEG = negative; NT = not tested; POS = positive; XDS = Xenobiotic Detection Systems, Inc.

[a]Number in parentheses represents test results (POS, NEG, or I) over the total number of acceptable trials.

5.2 Accuracy Analysis of the BG1Luc ER TA Agonist Data

The accuracy analysis using the 35 ICCVAM reference substances that produced a definitive BG1Luc ER TA result in agonist testing indicated a concordance of 97% (34/35), sensitivity of 96% (27/28), specificity of 100% (7/7), false positive rate of 0% (0/7), and false negative rate of 4% (1/28), **Table 5-4**.

Table 5-4 Accuracy of the BG1Luc ER TA Agonist Data

N	Accuracy	Sensitivity	Specificity	False Positive Rate	False Negative Rate
35[a]	97% (34/35)	96% (27/28)	100% (7/7)	0% (0/7)	4% (1/28)

Abbreviations: N = number

[a] A total 42 substances were evaluated in the BG1Luc ER TA Agonist test method. Seven substances did not produce a consensus classification and were omitted, leaving 35 substances for analysis.

5.2.1 Discordant Results for Agonist Analysis

Among the 35 substances used to calculate accuracy statistics, only L-thyroxine was a false negative in the BG1Luc ER TA when compared to the ICCVAM reference classification, **Table 5-5**. This Phase 4 substance was tested a single time in one laboratory, XDS. This substance is classified as Positive (2/3) by ICCVAM based on two reports of positive agonist activity and one report of no agonist activity. The two positive results were in GH3 cells (rat pituitary adenoma) (Fujimoto et al. 2004) and HeLa cells (human cervical carcinoma) (Takeyoshi 2006), whereas MCF-7 cells (human breast adenocarcinoma) (Fujimoto et al. 2004) showed no estrogenic response when exposed to L-thyroxine. These reports indicate a possible tissue-specific response to this chemical, which may explain the lack of ER agonist activity observed in this experiment with BG-1 cells (human ovarian carcinoma).

Table 5-5 Discordant Substance in the BG1Luc ER TA Agonist Test Method

Substance	CASRN	MESH Chemical Class	Product Class	BG1Luc ER TA Classification	ICCVAM Reference Classification
L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	NEG	POS

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; MeSH = U.S. National Library of Medicine's Medical Subject Headings; N = number

5.3 Accuracy Analysis of the BG1Luc ER TA Antagonist Test Method

Accuracy analysis conducted with the 25 reference substances that produced a definitive result in antagonist testing indicated an overall accuracy of 100% (25/25), sensitivity of 100% (3/3), specificity of 100% (22/22), false positive rate of 0% (0/22), and false negative rate of 0% (0/3), **Table 5-6**.

Table 5-6 Accuracy of the BG1Luc ER TA Antagonist Test Method

N	Accuracy	Sensitivity	Specificity	False Positive Rate	False Negative Rate
25[a]	100% (25/25)	100% (3/3)	100% (22/22)	0% (0/22)	0% (0/3)

Abbreviations: N = number

[a]A total 25 substances were evaluated in the BG1Luc ER TA Antagonist test method.

5.4 Comparison of BG1Luc ER TA Results with CERI STTA (US EPA OPPTS 890.1300).

The CERI STTA (OECD 2009; Takeyoshi 2006) method for assessing ER-alpha agonist activity of test substances is currently the only ER TA test method accepted by regulatory agencies. This test system utilizes the hERα-HeLa-9903 cell line, which is derived from a human cervical tumor, with two stably inserted constructs: the hERα expression construct (encoding the full-length human receptor), and a firefly luciferase reporter construct bearing five tandem repeats of a vitellogenin Estrogen-Responsive Element (ERE) driven by a mouse metallothionein (MT) promoter TATA element. Because the BG1Luc ER TA is another STTA that could be considered for regulatory use, a comparison of test method accuracy between these two test methods was conducted based on a list of ICCVAM-recommended agonist reference substances for which definitive classifications have been produced in both methods. These substances are listed in **Table 5-7**. These results show identical levels of accuracy when both methods tested the same agonist reference chemicals; concordance 95% (25/26), sensitivity 95% (21/22), and specificity 100% (4/4), **Table 5-8** and **Table 5-9**. The test methods differed only in the one false negative from each method; L-thyroxine was false negative in BG1Luc ER TA and *p*-n-nonylphenol was false negative in CERI ER TA. Overall, these data suggest a very high level of agreement in the performance of these two assays.

Table 5-7 Substances used in the Evaluation of Accuracy of the BG1Luc ER TA and CERI ER TA Test Method Results

Substance	CASRN	ICCVAM Reference Classification	BG1	CERI[a]
17ß-Estradiol	50-28-2	POS	POS	POS
17a-Estradiol	57-91-0	POS	POS	POS
17a-Ethinyl estradiol	57-63-6	POS	POS	POS
4-Cumylphenol	599-64-4	POS	POS	POS

Substance	CASRN	ICCVAM Reference Classification	BG1	CERI[a]
4-tert-Octylphenol	140-66-9	POS	POS	POS
Apigenin	520-36-5	POS	POS	POS
Atrazine	1912-24-9	NEG	NEG	NEG
Bisphenol A	80-05-7	POS	POS	POS
Bisphenol B	77-40-7	POS	POS	POS
Butylbenzyl phthalate	85-68-7	POS	POS	POS
Corticosterone	50-22-6	NEG	NEG	NEG
Coumestrol	479-13-0	POS	POS	POS
Daidzein	486-66-8	POS	POS	POS
Diethylstilbestrol	56-53-1	POS	POS	POS
Estrone	53-16-7	POS	POS	POS
Ethyl paraben	120-47-8	POS	POS	POS
Genistein	446-72-0	POS	POS	POS
Kaempferol	520-18-3	POS	POS	POS
Kepone	143-50-0	POS	POS	POS
Linuron	330-55-2	NEG	NEG	NEG
L-thyroxine	51-48-9	POS	NEG	POS
Methyl testosterone	58-18-4	POS	POS	POS
Mifepristone	84371-65-3	NEG	NEG	NEG
Norethynodrel	68-23-5	POS	POS	POS
p-n-Nonylphenol	104-40-5	POS	POS	NEG
p,p'- Methoxychlor	72-43-5	POS	POS	POS
Spironolactone	52-01-7	NEG	NEG	NEG

Abbreviations: CASRN = Chemical Abstract Services Registry Number; CERI = the Chemicals Evaluation and Research Institute, Japan; I = inadequate; NEG = negative; nt = not tested; OECD = Organization for Economic Cooperation and Development; POS = positive;

[a]Data published by the Chemicals Evaluation and Research Institute, Japan (CERI) (Takeyoshi 2006)

Table 5-8 Accuracy of BG1Luc Test Method Assessed using Agonist Reference Chemicals Listed in Table 5-5

		BG1Luc ER TA Agonist Classification		
		POS	**NEG**	**Total**
ICCVAM Consensus Classification	**POS**	21	1	22
	NEG	0	4	4
	Total	21	5	26

Concordance 96% (25/26)

Sensitivity 95% (21/22)

Specificity 100% (4/4)

Table 5-9 Accuracy of CERI ER TA Test Method Assessed Using Agonist Reference Chemicals Listed in Table 5-5

		CERI ER TA Classification		
		POS	**NEG**	**Total**
ICCVAM Consensus Classification	**POS**	21	1	22
	NEG	0	4	4
	Total	21	5	26

Concordance 96% (25/26)

Sensitivity 95% (21/22)

Specificity 100% (4/4)

5.5 Comparison of BG1Luc ER TA EC_{50} and IC_{50} Values with Values From ICCVAM Reference Data

Although the primary goal of the BG1Luc ER TA is to provide a qualitative assessment of estrogenic/anti-estrogenic activity, quantitative measures of activity (i.e., EC_{50} and IC_{50} values) are usually obtained for positive results. EC_{50} and IC_{50} values obtained from BG1Luc ER TA test results were compared to median values from other ER TA test methods reported in the literature. The substances used for these comparisons are listed in **Table 5-10** for EC_{50} and **Table 5-11** for IC_{50} comparisons. Regression analyses of these data are presented in **Figures 5-1** and **5-2**, respectively.

Based on EC_{50} values obtained for 26 substances, the correlation coefficient between the Log EC_{50} for the BG1Luc ER TA agonist test method and that reported for other ER TA test methods reported in the literature was $R^2 = 0.839$. This relatively high correlation indicates that the BG1Luc ER TA agonist test method might be considered for quantitative as well as qualitative assessment of estrogenic activity.

Likewise, based on IC_{50} values obtained for 3 substances, the correlation coefficient between the Log IC_{50} for the BG1Luc ER TA antagonist test method and that reported for other ER TA test methods reported in the literature was $R^2 = 0.95$. Again, this high correlation suggests that the BG1Luc ER TA might also be considered for quantitative as well as qualitative assessment of anti-estrogenic activity. However, this conclusion is necessarily limited by the small number of substances (n=3) upon which it is based.

Table 5-10 List of Median EC_{50} Values Substances For Substances used to Generate EC_{50} Linear Regression

Substance Name	BG1Luc ER TA Median EC_{50} (M)	ICCVAM Reference Data Median EC_{50} (M)
17α-Estradiol	3.02×10^{-10}	5.20×10^{-09}
17α-Ethinyl estradiol	7.09×10^{-12}	5.20×10^{-11}
17β-Estradiol	3.37×10^{-12}	8.65×10^{-11}
19-Nortestosterone	1.65×10^{-06}	2.00×10^{-07}
4-Cumylphenol	3.03×10^{-07}	3.22×10^{-07}
4-*tert*-Octylphenol	2.08×10^{-08}	1.00×10^{-07}
5α-Dihydrotestosterone	8.97×10^{-08}	1.33×10^{-07}
Apigenin	1.40×10^{-06}	7.65×10^{-07}
Bisphenol A	3.95×10^{-07}	5.00×10^{-07}
Bisphenol B	2.36×10^{-07}	9.20×10^{-08}
Coumestrol	1.31×10^{-07}	1.60×10^{-08}
Daidzein	6.75×10^{-07}	4.90×10^{-07}
Dicofol	2.22×10^{-06}	7.05×10^{-06}
Diethylstilbestrol	2.08×10^{-11}	6.60×10^{-11}
Estrone	2.16×10^{-10}	2.10×10^{-09}
Fenarimol	9.15×10^{-06}	7.00×10^{-06}
Genistein	3.00×10^{-07}	6.75×10^{-08}
Kaempferol	2.55×10^{-07}	1.60×10^{-07}
meso-Hexestrol	1.62×10^{-11}	1.00×10^{-10}
Methyl testosterone	6.49×10^{-07}	1.58×10^{-08}
Norethynodrel	1.26×10^{-07}	6.40×10^{-09}
o,p'-DDT	4.22×10^{-07}	1.69×10^{-06}
p,n-Nonylphenol	2.50×10^{-06}	3.60×10^{-07}

Substance Name	BG1Luc ER TA Median EC_{50} (M)	ICCVAM Reference Data Median EC_{50} (M)
p,p'-Methoxychlor	8.43×10^{-07}	5.25×10^{-06}
Tamoxifen	6.73×10^{-08}	5.30×10^{-07}

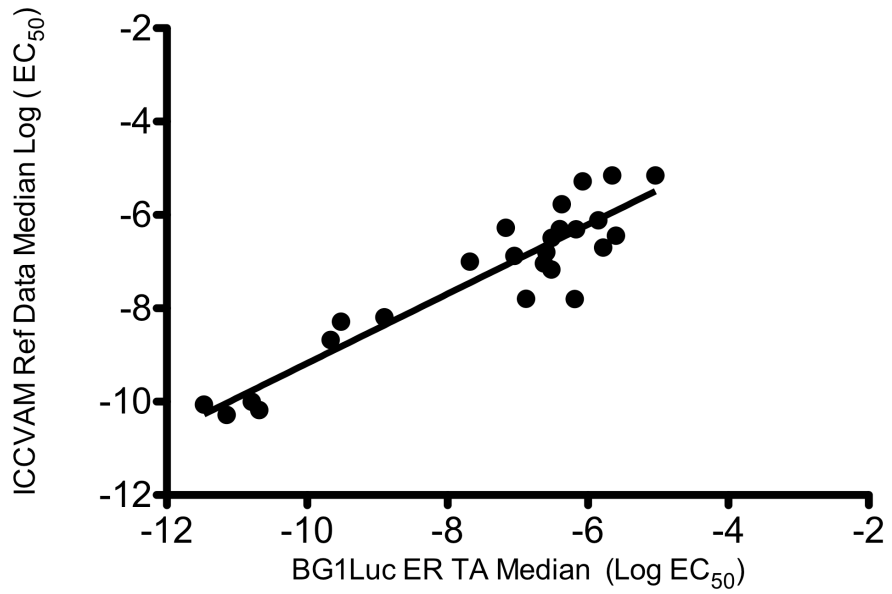
Abbreviations: EC_{50} = half -maximal effective concentration; M = molar

Table 5-11 List of Median IC_{50} Values Substances For Substances used to Generate IC_{50} Linear Regression

Substance Name	BG1Luc ER TA Median IC_{50} (M)	ICCVAM Reference Data Median IC_{50} (M)
4-Hydroxytamoxifen	4.94×10^{-09}	2.13×10^{-09}
Raloxifene HCl	1.24×10^{-09}	2.31×10^{-09}
Tamoxifen	7.12×10^{-07}	4.00×10^{-07}

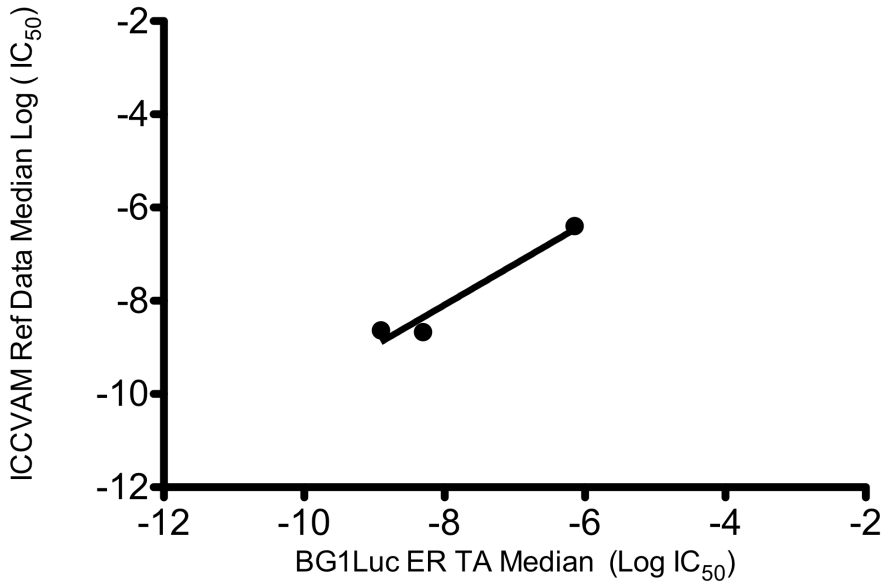
Abbreviations: IC_{50} = concentration of test substance inhibiting the reference estrogen by 50%; M = molar

Figure 5-1 Relationship of EC_{50} Values Obtained in the BG1Luc ER TA versus EC_{50} Values from ICCVAM Reference Data



Each point in this figure represents a median EC_{50} value obtained in the BG1Luc ER TA compared with the median ICCVAM EC_{50} value (from the 2010 updated reference data).

Figure 5- 2 Relationship of IC_{50} Values Obtained in the BG1Luc ER TA versus IC_{50} Values from ICCVAM Reference Data



Each point in this figure represents a median IC_{50} value obtained in the BG1Luc ER TA compared with the median ICCVAM IC_{50} value (from the 2010 updated reference data)

5.6 Concordance of BG1Luc ER TA Results with Estrogen Receptor Binding

Results from the BG1Luc ER TA were examined for concordance with published reports of ER binding. ER binding results from the list of the 34 reference substances used for this analysis along with agonist and antagonist test results from the BG1Luc ER TA are provided in **Table 5-12**. Because results in binding studies only indicate the ability to bind the ER receptor, and therefore do not distinguish between agonist or antagonist activity, a positive result in BG1Luc ER TA for either the agonist or antagonist activity was considered "Positive" in the concordance analysis provided in **Table 5-13**. There was 97% (33/34) concordance between the BG1Luc ER TA and ER binding data from the literature. The single discordant test substance was medroxy-progesterone acetate (MPA), which was positive in the ER TA antagonist assay but was reported in two published studies as negative for ER binding. MPA was tested a single time during Phase 4 at one participating laboratory XDS, which reported an IC_{50} of 5.0×10^{-5} M. In light of the excellent degree of agreement between ER binding and BG1Luc ER TA (with no false negative results), it appears that evaluating results from BG1Luc ER TA agonist and antagonist testing would provide a viable alternative to conducting ER binding studies. This cannot currently be accomplished with the only accepted ER TA method due to the inability of the CERI STTA method to assess ER antagonist activity.

193 **Table 5-12 Substances Used for Assessing Concordance with ER Binding**

Substance	CASRN	BG1 Agonist Classification	BG1 Antagonist Classification	Overall BG1 Classification	ER Binding Classification (Literature)
17ß-Estradiol	50-28-2	POS	NEG	POS	POS
17α-Estradiol	57-91-0	POS	I	POS	POS
17α-Ethinyl estradiol	57-63-6	POS	NEG	POS	POS
2-*sec*-Butylphenol	89-72-5	POS	NEG	POS	POS
4-Cumylphenol	599-64-4	POS	NEG	POS	POS
4-Hydroxytamoxifen	68047-06-3	NEG	POS	POS	POS
4-*tert*-Octylphenol	140-66-9	POS	NEG	POS	POS
Apigenin	520-36-5	POS	NEG	POS	POS
Bisphenol A	80-05-7	POS	NEG	POS	POS
Bisphenol B	77-40-7	POS	NEG	POS	POS
Butylbenzyl phthalate	85-68-7	POS	NEG	POS	POS
Corticosterone	50-22-6	NEG	NEG	NEG	NEG
Coumestrol	479-13-0	POS	NEG	POS	POS
Daidzein	486-66-8	POS	NEG	POS	POS
Dicofol	115-32-2	POS	NEG	POS	POS
Diethylstilbestrol	56-53-1	POS	NEG	POS	POS
Estrone	53-16-7	POS	NEG	POS	POS
Ethyl paraben	120-47-8	POS	NEG	POS	POS
Fenarimol	60168-88-9	POS	NEG	POS	POS
Genistein	446-72-0	POS	NEG	POS	POS
Kaempferol	520-18-3	POS	NEG	POS	POS
Kepone	143-50-0	POS	NEG	POS	POS
L-thyroxine	51-48-9	NEG	NEG	NEG	NEG
Medroxy-progesterone acetate	71-58-9	NEG	POS	POS	NEG
meso-hexestrol	84-16-2	POS	NEG	POS	POS
Mifepristone	84371-65-3	NEG	NEG	POS	POS
Morin	480-16-0	POS	NEG	POS	POS
Norethynodrel	68-23-5	POS	NEG	POS	POS
o,p'-DDT	789-02-6	POS	NEG	POS	POS
p-n-Nonylphenol	104-40-5	POS	NEG	POS	POS
p,p'-Methoxychlor	72-43-5	POS	NEG	POS	POS
Phenolphthalin	81-90-3	POS	NEG	POS	POS
Raloxifene HCl	82640-04-8	NEG	POS	POS	POS

Substance	CASRN	BG1 Agonist Classification	BG1 Antagonist Classification	Overall BG1 Classification	ER Binding Classification (Literature)
Tamoxifen	10540-29-1	I	POS	POS	POS

Abbreviations: BG1 = BG1Luc ER TA; CASRN = Chemical Abstracts Service Registry Number; I = indequate; NEG = negative; POS = positive

Table 5-13 Concordance of BG1Luc ER TA Test Method Results Compared with ER Binding

		BG1 Classification		
		POS	**NEG**	**Total**
ER Binding	**POS**	31	0	31
	NEG	1	2	3
	Total	32	2	34

Concordance 97% (33/34)

5.7 Comparison of BG1Luc ER TA Test Method Results with Uterotrophic Assay Results

Results from the BG1Luc ER TA were examined for concordance with published data from the uterotrophic assay (Owens and Ko√´ter 2003). Data from the uterotrophic assay was available for 13 substances tested in the BG1Luc ER TA agonist test method **(Table 5-14)**. Based on a comparison with the *in vivo* uterotrophic assay classification, the 13 substances with conclusive test results in the BG1Luc ER TA agonist test method produced overall concordance of 92% (12/13), **Table 5-15**. All substances found positive in the uterotrophic assay were also positive in the BG1Luc ER TA method. The only discordant substance, butylbenzyl phthalate, was positive for ER agonist activity in the BG1Luc ER TA agonist test method and negative in the uterotrophic assay. These data indicate that the BG1Luc ER TA agonist test method has very good agreement with the *in vivo* results obtained with the uterotrophic assay, with no false negative results.

Table 5-14 Substances Used in the Comparison of BG1Luc Agonist Classification and *In Vivo* Uterotrophic Assay Data

ICCVAM Reference Substance	CASRN	BG1Luc ER TA Agonist Classification	Overall Uterotrophic Assay Study Data	OECD Study Uterotrophic Assay Data[a]	CERI Study Uterotrophic Assay Data[b]
17-α Estradiol	57-91-0	POS	POS	nt	POS
17-α Ethinyl estradiol	57-63-6	POS	POS	POS	POS
4-*tert*-Octylphenol	140-66-9	POS	POS	nt	POS
4-Cumylphenol	599-64-4	POS	POS	nt	POS
Bisphenol A	80-05-7	POS	POS	POS	POS
Bisphenol B	77-40-7	POS	POS	nt	POS
Butylbenzyl phthalate	85-68-7	POS	NEG	NEG	NEG
Daidzein	486-66-8	POS	POS	nt	POS
Estrone	53-16-7	POS	POS	nt	POS
Genistein	446-72-0	POS	POS	POS	POS
Ketoconazole	65277-42-1	NEG	NEG	nt	NEG
Methyl Testosterone	58-18-4	POS	POS	nt	POS
o.p'-DDT	789-02-6	POS	POS	POS	nt

Abbreviations: CASRN = Chemical Abstract Services Registry Number; CERI = the Chemicals Evaluation and Research Institute, Japan; NEG = negative; nt = not tested; OECD = Organization for Economic Cooperation and Development; POS = positive;

[a]Pooled data from the validation of the OECD Uterotrophic Bioassay (Kanno et al. 2003a, 2003b; Owens and Ashby 2002)

[b]Data published by the Chemicals Evaluation and Research Institute, Japan (CERI), as part of comparison database of ER TA and uterotrophic data (Takeyoshi 2006).

Table 5-15 Concordance of BG1Luc ER TA Agonist Classification and *In Vivo* Uterotrophic Assay Data

		BG1Luc ER TA Agonist Classification		
		POS	**NEG**	**Total**
***In Vivo* Uterotrophic Data**	**POS**	11	0	11
	NEG	1	1	2
	Total	12	1	13

Concordance 92% (12/13)

Fujimoto N, Jinno N, Kitamura S. 2004. Activation of estrogen response element dependent transcription by thyroid hormone with increase in estrogen receptor levels in a rat pituitary cell line, GH3. J Endocrinol 181(1): 77-83.

Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens JW. 2003a. The OECD program to validate the rat uterotrophic bioassay. Phase 2: Coded single-dose studies. Environmental Health Perspectives 111(12): 1550-1558.

Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens JW. 2003b. The OECD program to validate the rat uterotrophic bioassay. Phase 2: Dose-response studies. Environmental Health Perspectives 111(12): 1530-1549.

OECD. 2009. Test No. 455. Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals [adopted 7 September 2009]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health EffectsOECD Publishing. Available: http://dx.doi.org/10.1787/9789264076372-en [accessed 30 July 2010].

Owens JW, Ashby J. 2002. Critical review and evaluation of the uterotrophic bioassay for the identification of possible estrogen agonists and antagonists: In support of the validation of the OECD uterotrophic protocols for the laboratory rodent. Crit Rev Toxicol 32(6): 445-520.

Owens JW, Ko√′ter HBWM. 2003. The OECD program to validate the rat uterotrophic bioassay: An overview. Environmental Health Perspectives 111(12): 1527-1529.

Takeyoshi M. 2006. Draft Report of Pre-validation and Inter-laboratory Validation For Stably Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line: Chemicals Evaluation and Research Institute (CERI), Japan.